TABLE I

Precision of the method for measurement of plasma concentrations of phenylpropanolamine determined as the coefficient of variation of the mean of five replicate assays.

Plasma	Phenylpropanolamine Concentration (ng/ml)	N	Area Ratio	Mean	Standard Deviation	Coefficient of Variation
	5.23		0.0811			
	5.23		0.0926			
	5.23	4	0.0850	0.0868	0.0049	±5.63%
	5.23		0.0883			
	20.94		0.2808			
	20.94		0.2806			
	20.94	5	0.2770	0.2812	0.0050	±1.80%
	20.94		0.2778			
	20.94		0.2897			
	104.70		1.4860			
	104.70		1.2987			
	104.70	4	1.364	1.3619	0.0883	±6.48%
	104.70		1.2987			
	157.05		1.9904			
	157.05		1.9534			
	157.05	5	1.9580	1.9616	0.0322	±1.64%
	157.05		1.9139			
	157.05		1.9930			
	261.75		3.2536			
	261.75		3.1621			
	261.75	5	3.1930	3.1852	0.0520	±1.63%
	261.75		3.2039			
	261.75		3.1132			

TABLE Ia

Precision of the method for measurement of urine concentrations of phenylpropanolamine determined as the coefficient of variation of the mean of five replicate assays.

Urine	Phenylpropanolamine Concentration (µg/ml)	Area Ratio	Mean	Standard Deviation	Coefficient of Variation
	0.955	0.01529			
	0.955	0.01551			
	0.955	0.01502	0.01506	0.00034	±2.23%
	0.955	0.01471			
	0.955	0.01479			
	3.82	0.06072			
	3.82	0.06085			
	3.82	0.06106	0.06094	0.00034	±0.55%
	3.82	0.06060			
	3.82	0.06146			
	9.55	0.1566			
	9.55	0.1557			
	9.55	0.1560	0.1556	0.00064	±0.41%
	9.55	0.1563			
	9.55	0.1546			
	38.2	0.6411			
	38.2	0.6526			
	38.2	0.6475	0.6475	0.0041	±0.64%
	38.2	0.6486			
	38.2	0.6478			
	95.5	1.6369			
	95.5	1.6303			
	95.5	1.6305	1.6324	0.0031	±0.19%
	95.5	1.6345			
	95.5	1.6298			

TABLE II

Reproducibility and accuracy of plasma assay. Plasma was spiked with 99.43 ng/ml phenylpropanolamine HCl, then separated into five separate aliquots which were stored at -20 C. These aliquots were assayed on five separate days over a 97 day period.

Phenylpropanolamine HCl Determined (ng/ml)	Average (ng/ml) and Coefficient of Variation
97.12	
102.34	
87.12	93.09($\pm$7.03%, n = 5)
87.55	
91.33	
	Percent difference between actual plasma level and the average determined level
	6.37

Table III

Reproducibility and accuracy of urine assay. Urine was spiked with 22.72 µg/ml phenylpropanolamine HCl, then separated into 8 separate aliquots which were stored at -20°C. These aliquots were assayed two at a time on four different days over a two week period.

Phenylpropanolamine HCl Determined (µg/ml)	Average (µg/ml) and Coefficient of Variation
22.73	
22.02	
21.65	
21.45	22.36 (±2.69%, n = 8)
22.47	
22.93	
23.13	
22.48	
	Percent difference between actual urine level and the average determined level: 1.58

Table IV

Reproducibility of detector response. The same extracts of phenylpropanolamine from plasma and from urine were injected into the GLC (for plasma) and the H.P.L.C. (for urine) five times on the same day.

Plasma	Phenylpropanolamine HCl Concentration (ng/ml)	Area Ratio	Mean	Standard Deviation	Coefficient of Variation
	104.70	1.3605			
	104.70	1.3636			
	104.70	1.3778	1.3652	0.0072	+0.53%
	104.70	1.3605			
	104.70	1.3636			

Urine	Phenylpropanolamine HCl Concentration (µg/ml)	Area Ratio	Mean	Standard Deviation	Coefficient of Variation
	22.72	0.2676			
	22.72	0.2706			
	22.72	0.2707	0.2708	0.00203	±0.75%
	22.72	0.2727			
	22.72	0.2724			

Table V

Stability of phenylpropanolamine HCl in plasma. Plasma was spiked with phenylpropanolamine HCl at three levels (approximately 20, 100, and 190 ng/ml), then separated into separate aliquots which were stored in silicone coated 10 ml blood collection tubes (B-D Vacutainer Brand) at -20°C. Aliquots were assayed periodically over a 33 day period.

Day	Phenylpropanolamine HCl Determined (ng/ml)		
0	21.5	106.2	190.0
1	20.0	102.4	184.1
7	23.3	111.1	183.3
12	26.7	108.9	187.8
15	24.4	118.9	198.9
21	22.0	100.0	177.0
28	23.0	114.0	198.0
33	18.8	106.4	187.1
Average	22.5	108.5	188.3
Standard Deviation	2.5	6.2	6.3
Coefficient of Variation	+11.11%	±5.71%	±3.35%

Appendix II
(continued)

Table VI

Stability of phenylpropanolamine HCl in urine. Urine was spiked with phenylpropanolamine HCl at three levels (approximately 2, 25 and 50 µg/ml), then separated into separate aliquots which were stored in one quart polyethylene bottles (normally used for urine collection in clinical studies) at three different temperatures (room temperature, 4°C and -20°C). Aliquots of these solutions were assayed periodically over a 28 day period.

Phenylpropanolamine HCl Determined (µg/ml)

Day	Room Temp	4°C	-20°C	Room Temp	4°C	-20°C	Room Temp	4°C	-20°C
0	1.76	1.81	1.77	21.79	23.82	24.39	49.87	49.39	48.69
3	2.13	2.12	2.10	24.82	25.01	24.87	49.73	50.04	50.11
7	2.23	2.15	2.08	22.52	24.67	24.79	50.22	50.97	50.67
11	1.90	1.96	2.29	18.19	23.51	24.39	44.74	51.60	48.12
15	0.95	1.46	2.10	15.43	24.76	24.69	38.56	50.88	49.77
23	1.03	1.37	2.12	13.01	21.29	22.80	25.53	46.43	50.93
28	1.33	1.68	2.50	14.10	23.48	24.66	25.73	47.43	48.58

4:1615303:1M1CMG
3/24/82

Figure 1

Standard curve for assay of phenylpropanolamine in plasma, constructed by plotting peak area ratio of phenylpropanolamine to internal standard against concentration of phenylpropanolamine.

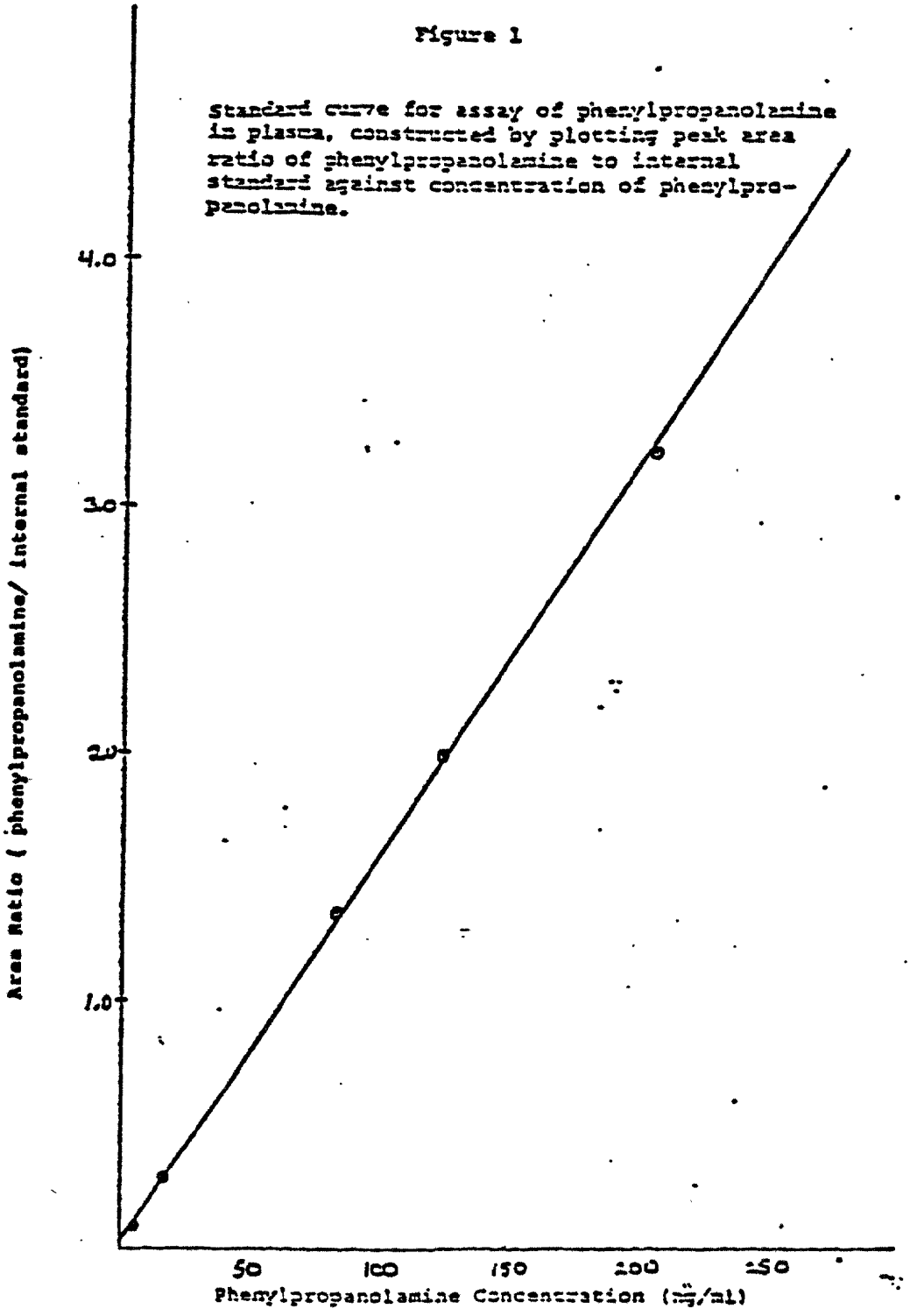


Figure 2

Standard curve for assay of phenylpropanolamine in urine, constructed by plotting peak area ratio of phenylpropanolamine to internal standard against concentration of phenylpropanolamine.

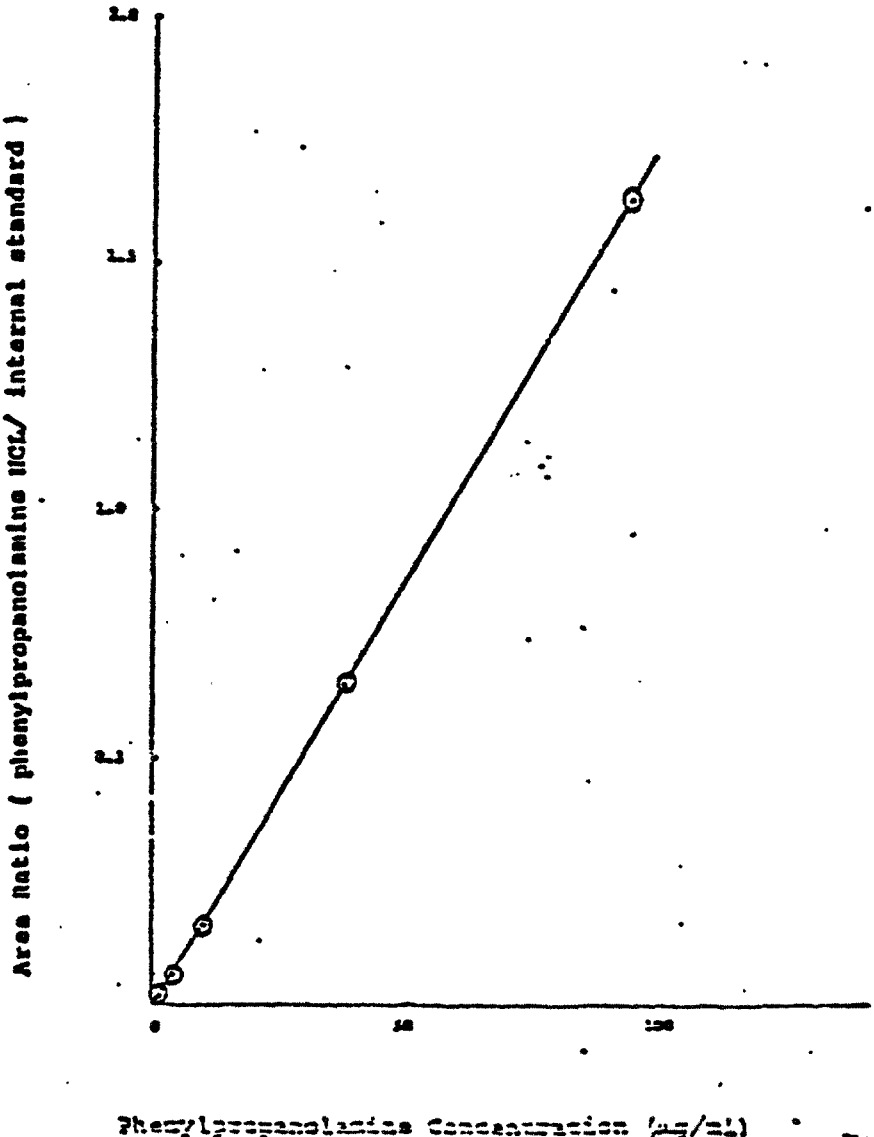


Figure 3

Chromatograms of (A) control extract of 1 ml plasma and (B) extract of 1 ml plasma containing 5.23 ng/ml of phenylpropanolamine HCL (1) and approximately 200 ng 2-amino-3-phenyl-1-propanol hydrochloride (2).

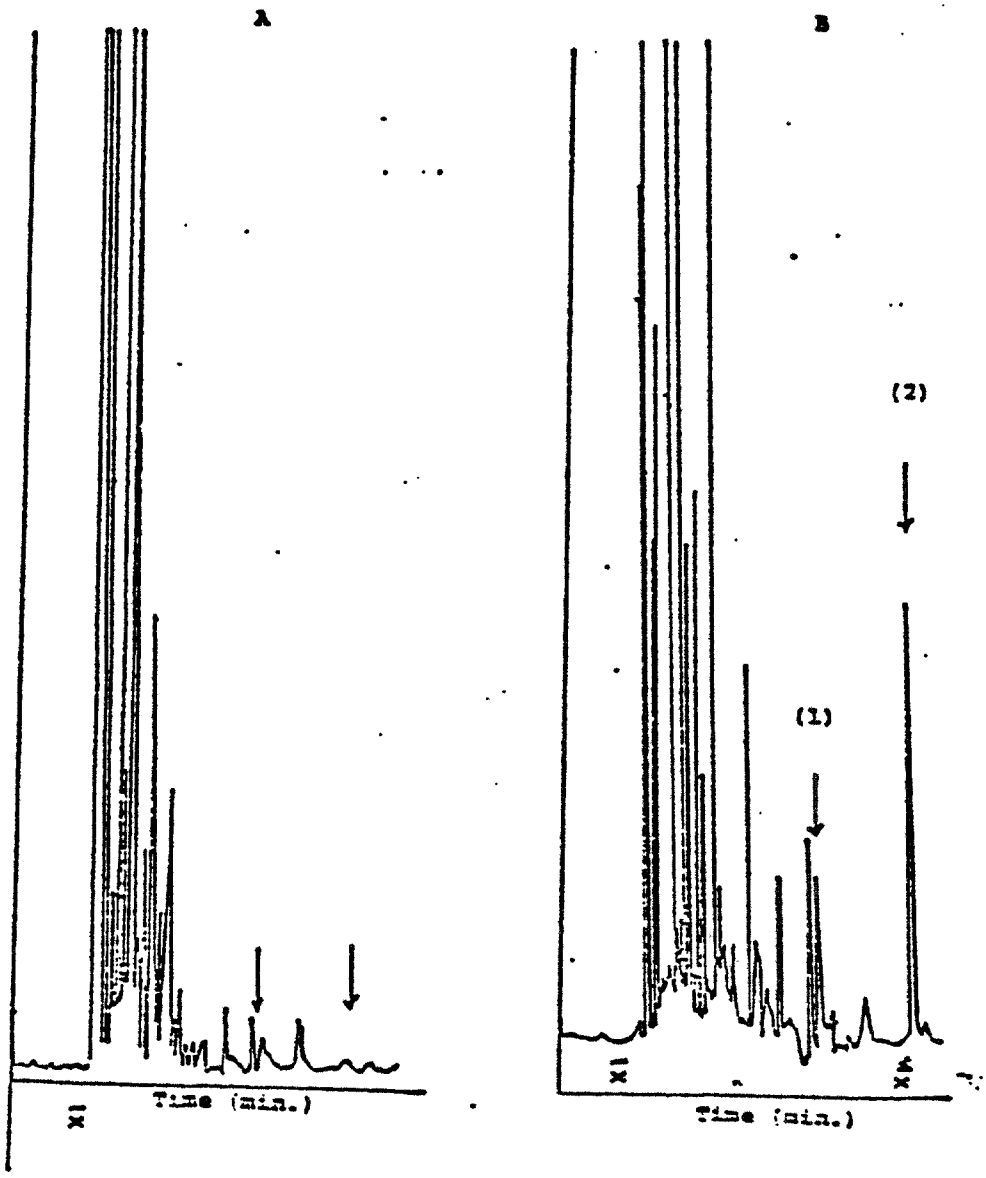


Figure 4

Chromatograms of (A) control sample of urine; and (B) control sample of urine containing 2.27µg/ml phenylpropanolamine HCL (1) and approximately 60µg amphetamine sulfate (2).

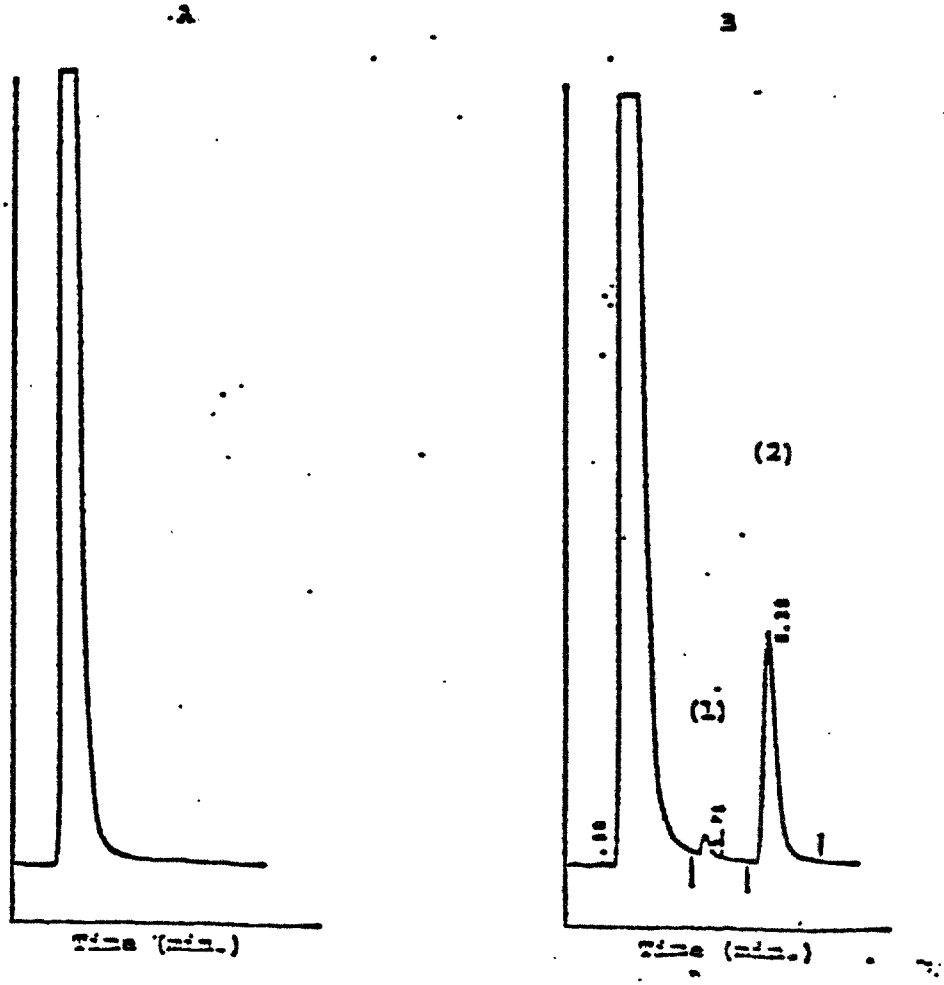
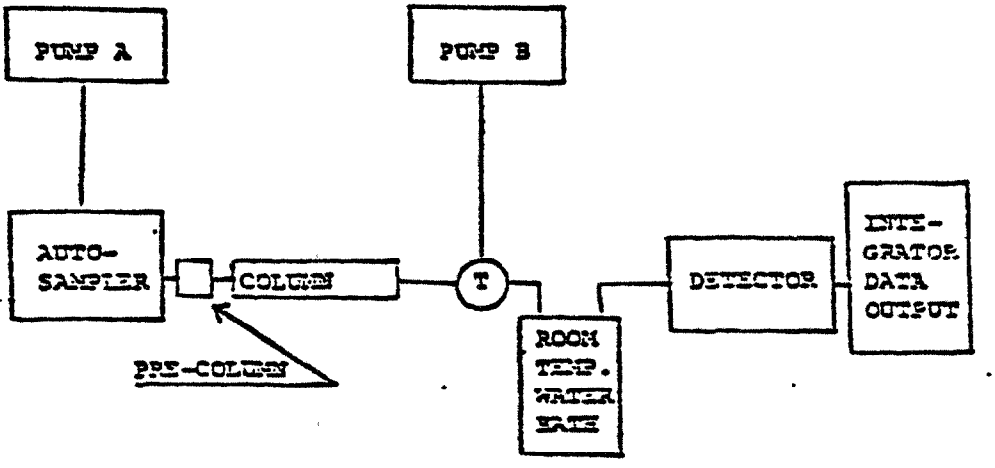


EXHIBIT 1



PUMP A: Mobile phase delivery at 1.5 ml/min.
PUMP B: Fluorepa® solution delivery at 1.5 ml/min.
AUTOSAMPLER: WISP 710A or equivalent
COLUMN: ODS-Hypersil, Shandon Southern
PRE-COLUMN: Water's Bondapak C_{18}/Corasil®
T: LC Teflon Tee joint
WATER BATH: 15'x0.027" coiled teflon tubing which serves as the in-line reactor is immersed in this room-temperature water bath
DETECTOR: Fluorometer, Schoeffel or equivalent
excitation at 340 nm
emission cutoff at 413 nm
INTEGRATOR: Spectra-Physics 4100 or equivalent

Assay of Residual PPA.HCl Content of GITS Recovered from Stools

INTRODUCTION

The following is a description of a high pressure liquid chromatographic method for the determination of phenylpropanolamine·HCl (PPA·HCl) content in OROS®. The determination involves crushing the systems and dissolving the particles in distilled water, and injecting a filtrate of this solution into the chromatographic system. The compound is resolved on a reverse phase column and detected by UV absorption at 254 nm. Quantification is obtained by linear regression analysis of peak areas of a standard curve containing at least three standard points. Results will be reported as the HCl salt of PPA. This assay will resolve PPA from α-aminopropiophenone.

SAMPLE PREPARATION

Accurately weigh (mg) each system, then place each system between two plastic weigh boats and crush with a rubber mallet. Quantitatively transfer the crushed system particles to a 250 ml volumetric flask and add about 100 ml distilled, deionized water. Place the volumetric flask in a sonic bath for 10 minutes, to dissolve the drug particles. Cool to room temperature, then fill each flask to volume with H_2O and mix. Filter a portion from each flask and inject 40 mcl into the chromatographic system.

STANDARD PREPARATION

For analysis of systems containing 75 mg of drug, accurately weigh about 60 mg PPA·HCl USP Reference Standard, or equivalent, and transfer quantitatively to a 50 ml volumetric flask.* Fill to volume with H_2O and mix. Prepare working standard dilutions by accurately pipeting the following volumes of PPA·HCl standard stock solution and H_2O into appropriate glass test tubes, and mix. Assuming 60 mg PPA·HCl was used to prepare the standard stock solution, the following calibration standards would be generated:

PPA·HCl Stock (ml)	H_2O (ml)	Final Volume (ml)	PPA·HCl (mg/ml)
1.00	5.00	6.00	0.200
1.00	3.00	4.00	0.300
1.00	2.00	3.00	0.400

Prepare standards daily prior to analysis.

*NOTE: For analysis of systems containing other than 75 mg of drug, divide the expected (labeled) system PPA·HCl content by 75. Then, multiply the product by 60 to get the amount of PPA·HCl needed to prepare a stock solution that, when diluted as suggested above, will bracket the expected sample concentration.

ANALYSIS

Assemble a liquid chromatograph employing a controlled volume pumping system, a sample injection device, a UV detector capable of detection at 254 nm and a suitable recorder and/or integrator. Use the chromatographic column as indicated.

EQUIPMENT

Pump:	Waters 6000 A or equivalent
Detector:	Waters M440 or equivalent
Injector:	Waters WISP 710 A or B Automatic Sample Processor, or Rheodyne 7105, or equivalent.
Column:	Waters Micro Bondapak C_{18} 10 micron or equivalent.
Recorder:	mV output matched to detector output
Integrator:	Spectra Physics 4100, or equivalent

OPERATING PARAMETERS

Flow Rate:	1.5 ml/min
Pressure:	2500 psig
Detector Wavelength:	254 nm
Chart Speed:	0.2 in/min or 0.5 cm/min
Injection Volume:	40 mcl
Column Temp:	Ambient
Attenuation:	0.05 AUFS
Retention Time:	PPA 7.4 min (nominal)

REAGENTS

Mobile Phase: 40:60 MeOH:buffer

Prepare as follows:

To a 1 liter volumetric flask add 700 ml distilled H_2O, 50 ml of 1 M NaH_2PO_4, pH 7, 1.9 g Hexane Sulfonate Na, and 20 ml of 0.25 M triethylammonium phosphate, pH 7.3: Fill to volume with H_2O and mix. Transfer contents to a 2 liter erlenmeyer flask and add 667 ml MeOH. Mix and degas by vacuum filtration.

COLUMN PERFORMANCE

Assemble the specified chromatographic system. To condition the column, set the monitoring wavelength and pass mobile phase through the column at the flow rate to be used for analysis. Equilibrate the system until a steady baseline is obtained and column pressure is stabilized. If repeated sample injections give a stable retention time, proceed to analyze the samples and record the actual conditions used for the analysis.*

*NOTE: If ∝-aminopropiophenone is to be quantified, inject an aliquot of a test mixture prepared by adding 0.1 ml of ∝-aminopropiophenone Stock Standard[1] to 9.9 ml of one of the PPA·HCl calibration standards. If a resolution factor of greater than 1 is obtained, proceed to analyze the sample preparations.

CALCULATIONS

IDENTITY

Identify the PPA peak (and, ∝-aminopropiophenone peak, if present) by comparison of the retention time of the sample preparation(s) with that of the

---

[1]Prepare a Stock Standard of ∝-aminopropiophenone·HCl as follows:

Weigh 25 mg of ∝-aminopropiophenone·HCl USP Reference Standard, or equivalent, and quantitatively transfer to a 50 ml volumetric flask. Dissolve and fill to volume with distilled water. If ∝-aminopropiophenone is detected in sample preparation(s), then dilute this Stock Standard with 0.05 M phosphate buffer, pH 6.5, to obtain 1,2,4, and 6 mcg/ml of ∝-aminopropiophenone·HCl working standards.

standard preparation(s). If the retention times match, sample peaks are identified.

CONCENTRATION

Construct a standard curve by plotting concentrations (mg/ml) of PPA·HCl vs. peak area on linear graph paper, or by calculating the best straight line by linear regression analysis. Measure the peak area of the Sample Preparations and determine the concentration of PPA·HCl in the samples from the standard curve. Then calculate:

A. $$\text{mg PPA·HCl in system} = C \times 250 \text{ ml}$$

B. $$\text{Wt \% PPA·HCl in system} = \frac{C \times 250 \text{ ml}}{W} \times 100\%$$ where

C = concentration of sample solution obtained from standard curve, in mg/ml

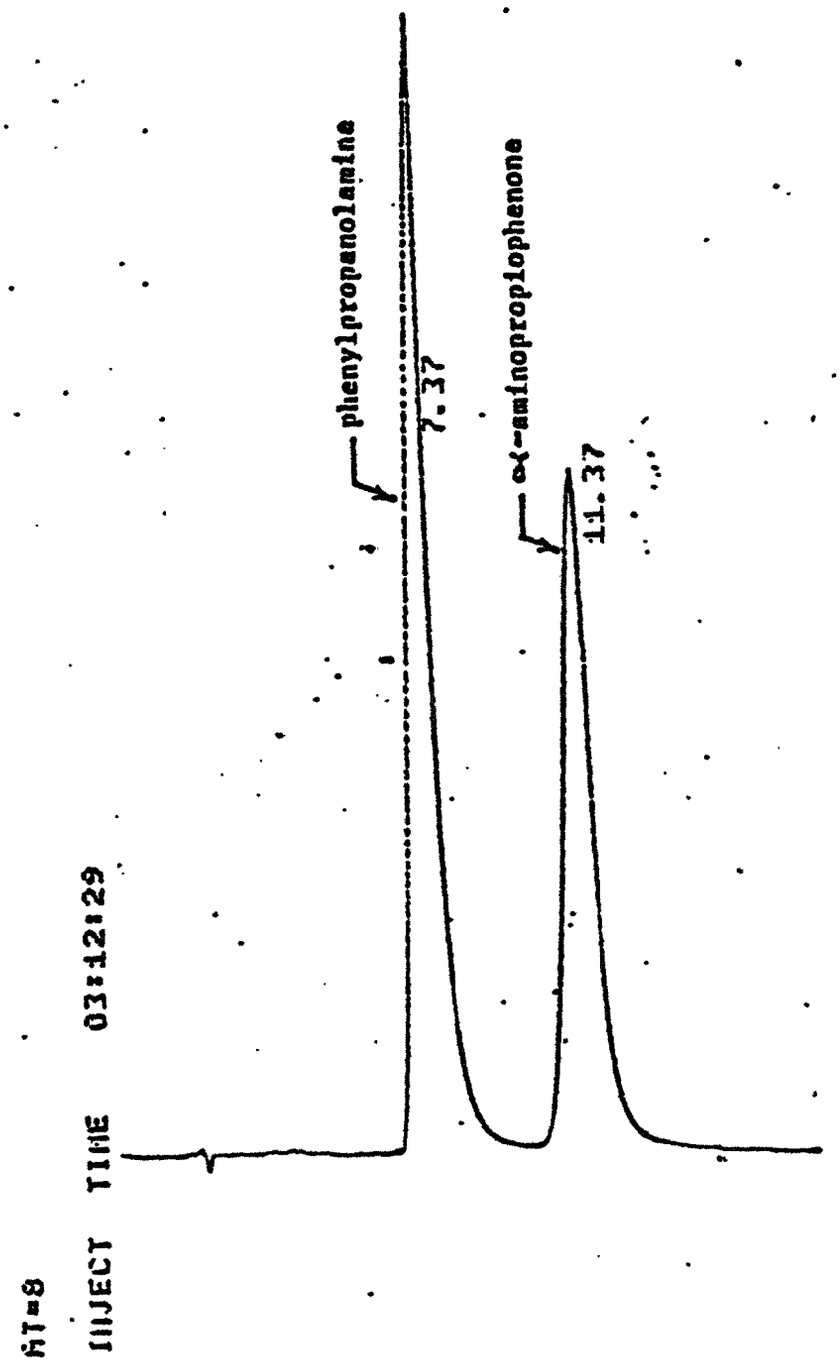
W = weight of system, in mg

C. From the individual assay results above, calculate the average drug content and standard deviation

NOTE: The same calculation may be used for quantifying ∝-aminopropiophenone using a standard curve obtained from the working standards suggested on pg. 3. Additional working standards may be prepared to bracket the detected concentration of ∝-aminopropiophenone in the sample preparation(s).

This method developed by Tom East

SAMPLE CHROMATOGRAM



PROTOCOL C-81-011: Study II

APPENDIX IV

Assay of Dosage Forms for PPA.HCl Content

Gastrointestinal Therapeutic Systems Control #154982

Run #	Mean	Range
1	75.1	69.5-81.4
2	75.4	67.8-80.3
3	74.5	64.7-78.8

Dexatrim Capsules Lot #SDF 282E

Sample #	MG PPA.HCl
1	87.2
2	78.2
3	63.5
4	79.7
5	80.7
6	64.7
7	69.1
8	61.0
9	86.3
10	66.2
Mean	73.7
±S.E.M.	3.1

Solution Lot #146082

Week	Sample #	mg PPA.HCl 37.5mg dose	mg PPA.HCl 25.0mg dose
1	1	37.56	25.28
	2	37.74	25.19
	3	37.55	25.16
	Mean	37.62	25.21
2	1	37.97	24.98
	2	37.87	24.98
	3	38.46	24.81
	Mean	38.10	24.92
4	1	37.95	25.33
	2	38.06	25.55
	Mean	38.01	25.44
5	1	37.59	25.14
	2	37.65	25.23
	Mean	37.62	25.19
7	1	37.85	25.29
	2	37.61	25.29
	Mean	37.73	25.29
8	1	37.71	25.17
	2	-*	25.08
	Mean	37.71	25.13

* Sample accidently destroyed during assay

PROTOCOL C-81-011: Study II

APPENDIX IV

Assay of Dosage Forms for PPA.HCl Content

Gastrointestinal Therapeutic Systems Control #154982

Run #	Mean	Range
1	75.1	69.5-81.4
2	75.4	67.8-80.3
3	74.5	64.7-78.8

Dexatrim Capsules Lot #SDF 282E

Sample #	MG PPA.HCl
1	87.2
2	78.2
3	63.5
4	79.7
5	80.7
6	64.7
7	69.1
8	61.0
9	86.3
10	66.2
Mean	73.7
±S.E.M.	3.1

Solution Lot #146082

Week	Sample #	mg PPA.HCl 37.5mg dose	mg PPA.HCl 25.0mg dose
1	1	37.56	25.28
	2	37.74	25.19
	3	37.55	25.16
	Mean	37.62	25.21
2	1	37.97	24.98
	2	37.87	24.98
	3	38.46	24.81
	Mean	38.10	24.92
4	1	37.95	25.33
	2	38.06	25.55
	Mean	38.01	25.44
5	1	37.59	25.14
	2	37.65	25.23
	Mean	37.62	25.19
7	1	37.85	25.29
	2	37.61	25.29
	Mean	37.73	25.29
8	1	37.71	25.17
	2	-*	25.08
	Mean	37.71	25.13

* Sample accidently destroyed during assay

Statistics Report
(ST-143-83)*

PHENYLPROPANOLAMINE ABSORPTION DURING ORAL ADMINISTRATION FROM GASTROINTESTINAL THERAPEUTIC SYSTEMS - STUDY 2

Elizabeth A. Leszczak

September 22, 1983

Distribution:

Mr. Richard Braun
Mr. Jerry Ostrov
Dr. Daivd Alkalay
Dr. Lewis Leeson
Ms. Elizabeth Leszczak
Statistics Files

*This report corrects results previously reported in Statistical Report ST-056-83, which it supersedes.

Statistics Report
(ST-143-83)

PHENYLPROPANOLAMINE ABSORPTION DURING ORAL ADMINISTRATION FROM GASTROINTESTINAL THERAPEUTIC SYSTEMS - STUDY 2

OBJECTIVE: To compare the bioavailability and the profiles for plasma levels and total urinary excretion for the following three oral dosage forms of phenylpropanolamine:

(1) Acutrim OROS capsules

(2) Dexatrim 12 hour sustained release capsules.

(3) Aqueous solution

DESIGN: Twelve subjects received 75 mg PPA HCL per day from one of the oral dosage forms indicated above, for four consecutive days during weeks one, three, and five of the study, according to a 3 x 3 Latin square design. Blood samples were drawn during days one and four of the dosing cycle and assayed for phenylpropanolamine HCL. Urine was collected during the entire dosing cycle.

STATISTICAL METHODS: The following parameters were analyzed by analysis of variance (Grizzle)[1]:

Area under the curve for day one

Area under the curve for day four

Total urinary excretion.

In addition, Westlake's confidence intervals[2] were calculated for each pair of dosage forms for these three parameters.

Plasma levels were also analyzed by a repeated measures analysis of variance (ANOVA)[3]. This analysis tests the null hypothesis of equality of all formulation means, as well as parallelism of the

response curves over time (formulation by time interaction). Comparisons between formulations at each time point were made using Student's t tests.

Since the ANOVA table for the repeated measures analysis contains three "error" terms (main plot error, subplot error, and the subject by time interaction), appropriate error terms for performing the tests at each time point were constructed as linear combinations of the main plot and subplot mean squares[4].

RESULTS AND CONCLUSIONS: There were no significant differences among the three oral dosage forms for bioavailability as measured by area under the curve at day four (p=0.12). For day one, the area under the curve for Dexatrim was significantly higher than both Acutrim and aqueous solution (p=0.036), but there was no significant difference between Acutrim and the aqueous solution (p>0.05). Significant differences in the shapes of the plasma concentration time curves are indicated by the highly significant formulation by time interaction (Table 2) and the plot of mean plasma levels (Figure 1 and 2). These differences can also be seen from the comparisons of the three formulations at each time point as presented in Table 1.

Elizabeth A Leszczak 9-23-83

Elizabeth A. Leszczak, M.S. Date
Statistician I

Approved: Murray Selwyn 9/23/83

Murray R. Selwyn, Ph.D. Date
Director,
Statistics and Data Systems

Records are on file and available for inspection in the offices of Research Statistics in Summit, New Jersey.

REFERENCES:

1. Grizzle, James E. "The Two-Period Changeover Design and Its Use in Clinical Trials", Biometrics 21, (June, 1965), pp. 467-480.

2. Westlake, W.J. "Use of Confidence Intervals in Analysis of Comparative Bioavailability Trials". J. Pharm. Sci. (1972) 61, pp. 1340-1341.

3. Westlake, W.J. "The Use of Balanced Incomplete Block Designs in Comparative Bioavailability Trials". Biometrics 30, (June, 1974). pp. 319-327.

4. Cochran, W.G. and Cox, G.M. Experimental Designs. Wiley (1957). pp. 298-299.

Table 1

Mean Plasma Concentration by Time*

Hour	Acutrim	Dexatrim	Aqueous Solution
0	0.0 a	0.0 a	0.0 a
0.5	24.1 a	4.9 b	34.8 a
1	45.5 a	44.0 a	75.3 b
2	64.7 a	95.1 b	104.0 b
3	71.3 a	119.9 b	96.9 c
4	66.7 a	141.4 b	81.0 a
6	77.3 a	154.1 b	60.2 a
8	73.6 a	117.2 b	41.9 c
12	70.7 a	79.7 a	19.7 b
16	61.4 a	42.1 b	98.4 c
24	18.5 a,b	7.6 a	28.7 b
48	20.8 a	9.1 a	13.6 a
72	26.1 a	8.7 a	14.0 a
73	63.9 a	49.2 a	54.7 a
74	84.6 a	101.2 a	83.5 a
76	84.4 a	136.9 b	80.4 a
77	92.7 a	152.9 b	143.6 b
78	90.0 a	149.5 b	134.7 b
80	89.4 a	131.1 b	109.2 c
81	90.8 a	125.7 b	158.0 c
82	87.6 a	104.0 a	152.2 b
84	94.0 a	77.0 a	116.6 b
88	64.6 a	43.1 b	60.1 a,b
96	21.8 a	7.6 a	12.5 a
100	10.2 a	3.0 a	5.5 a

*Means labeled with a common letter at each time point are not significantly different ($p>0.05$).

Table 2

Statistical Analysis for
Plasma Concentrations

ANOVA

Source	df	SS	MS	F	p
Subjects	11	159238	14476	6.07	0.003
Periods	2	9553	4777	2.00	0.16
Formulations	2	38603	19302	8.10	0.0003
Main plot error	20	47658	2383		
Times	24	1435450	59810	93.79	0.0001
Subject x Time	264	168349	638		
Formulation x Time	48	307529	6407	17.29	0.0001
Period x Time	48	18320	382	1.03	0.42
Subplot error	458	169739	371		

Table 3

Area Under the Curve - Day 1
Analysis of Variance

Source	df	SS	MS	F	P
Subjects	11	3000129	272739		
Periods	2	26343	13172	0.16	0.85
Formulations	2	648301	324151	3.99	0.036
Error	19	1542254	81171		

	Mean
Acutrim	1378
Dexatrim	1709
Aqueous Solution	1374

95% Westlake Confidence Limits

Acutrim vs Dexatrim	±31.4%
Acutrim vs Aqueous Solution	±17.7%
Dexatrim vs Aqueous Solution	±39.4%

Table 4

Area Under the Curve - Day 4
Analysis of Variance

Source	df	SS	MS	F	p
Subjects	11	6457559	587051		
Periods	2	600890	300445	2.49	0.11
Formulations	2	563923	281962	2.33	0.12
Error	20	2417374	120869		

	Mean
Acutrim	1666
Dexatrim	1808
Aqueous Solution	1972

95% Westlake Confidence Limits

Acutrim vs Dexatrim	±23.3%
Acutrim vs Aqueous Solution	±33.1%
Dexatrim vs Aqueous Solution	±22.6%

Figure 1

MEAN PLASMA CONCENTRATION OF PHENYLPROPANOLAMINE
DAY 1

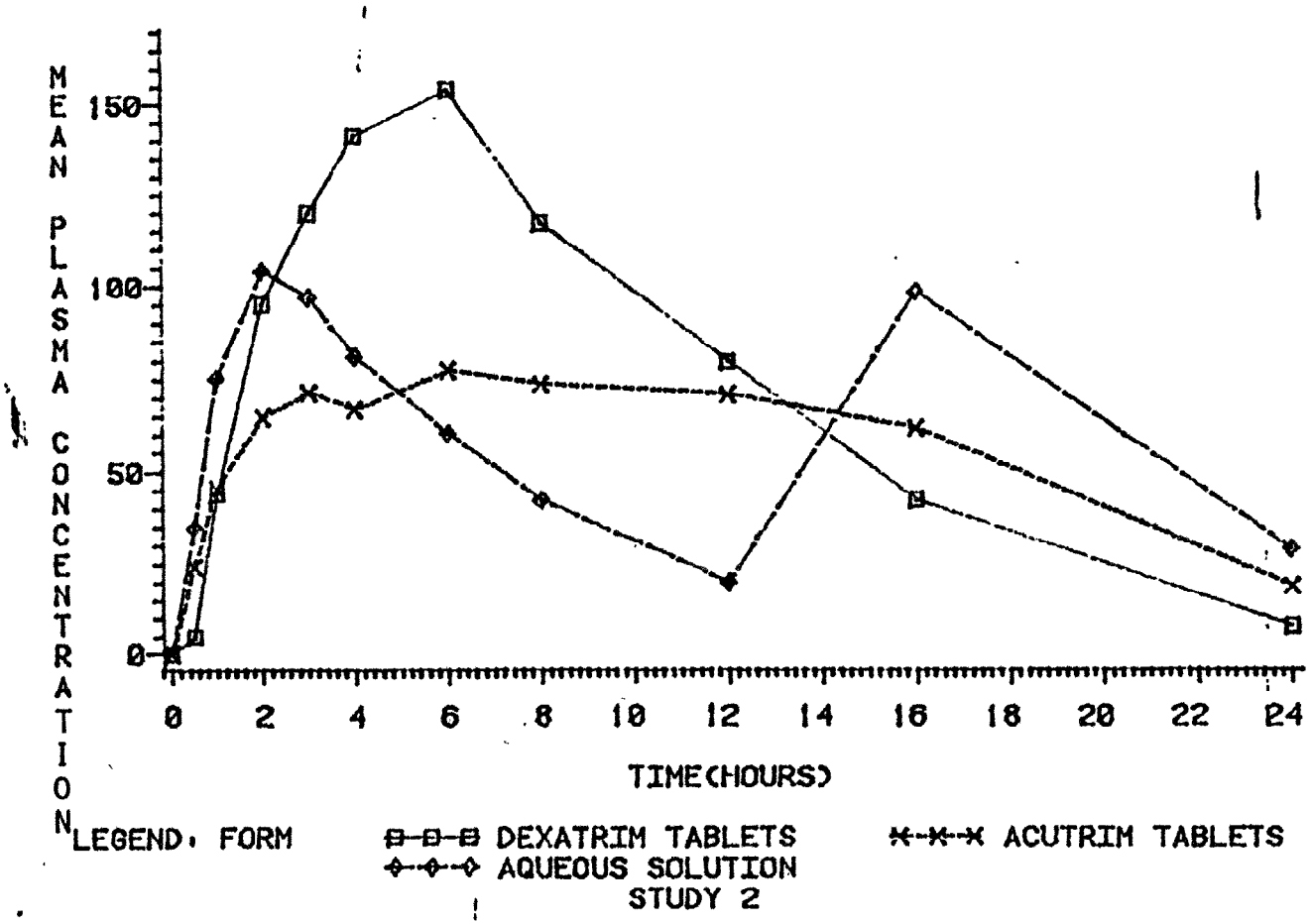
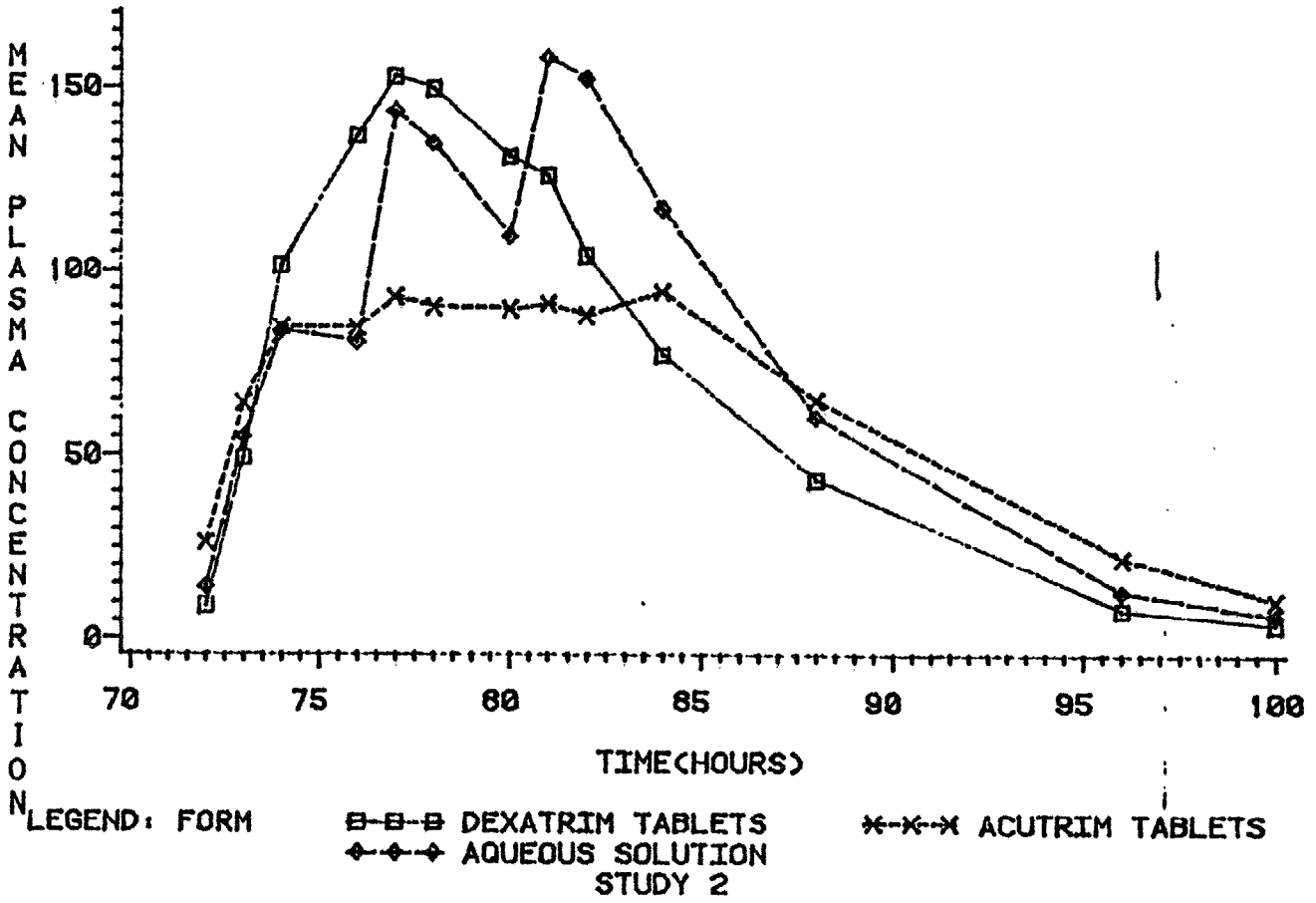


Figure 2

MEAN PLASMA CONCENTRATION OF PHENYLPROPANOLAMINE
DAY 4



Statistics Report
(ST-144-83)*

PHENYLPROPANOLAMINE ABSORPTION DURING ORAL ADMINISTRATION FROM GASTROINTESTINAL THERAPEUTIC SYSTEMS - STUDIES 1 AND 2

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September 26, 1983

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Statistics Files

*This report corrects results previously reported in Statistical Report ST-077-83, which it supersedes.

Statistics Report
(ST-144-83)

PHENYLPROPANOLAMINE ABSORPTION DURING ORAL ADMINISTRATION FROM GASTROINTESTINAL THERAPEUTIC SYSTEMS - STUDIES 1 AND 2

OBJECTIVE: To compare the bioavailability and the profiles for plasma levels for the following three oral dosage forms of phenylpropanolamine:

(1) Acutrim OROS capsules

(2) Dexatrim 12 hour sustained release capsules

(3) Aqueous solution

DESIGN: In study one, six subjects received 75 mg PPA HCL per day from one of the oral dosage forms indicated above, for four consecutive days during weeks one, three, and five of the study, according to a 3 x 3 Latin square design. Blood samples were drawn during day one, at 48 hours, and on day four of the dosing cycle and assayed for phenylpropanolamine HCL.

In the second study, twelve subjects were included. This study was essentially a replication of the first, although there were some minor differences in blood sampling times.

STATISTICAL METHODS: The following parameters were analyzed by analysis of variance (Grizzle)[1]:

Area under the curve for day one

Area under the curve for day four.

In addition, Westlake's confidence intervals[2] were calculated for each pair of dosage forms for these two parameters.

Plasma levels for those time points common to the two studies were also analyzed by a repeated measures analysis of variance

(ANOVA)[3,4]. This analysis tests the null hypothesis of equality of all formulation means, as well as parallelism of the response curves over time (formulation by time interaction). Comparisons between formulations at each time point were made using Student's t tests. Plasma values indicated as "<6.2" were taken to be zero in all analyses. Mean values for all time points are presented in Table 1 and Figures 1 through 3.

Since the ANOVA table for the repeated measures analysis contains several "error" terms, appropriate error terms for performing the tests at each time point were constructed as linear combinations of the main plot and subplot mean squares[5,6].

RESULTS AND CONCLUSIONS: There were no significant differences among the three oral dosage forms for bioavailability as measured by area under the curve at day one or day four ($p>0.05$). Significant differences in the shapes of the plasma concentration time curves are indicated by the highly significant formulation by time interaction (Table 2) and the plot of mean plasma levels (Figures 1, 2 and 3). These differences can also be seen from the comparisons of the three formulations at each time point as presented in Table 1.

Elizabeth A Leszczak 9-26-83

Elizabeth A. Leszczak, M.S. Date
Statistician I

Approved: Murray Selwyn 9/26/83

Murray S. Selwyn, Ph.D. Date
Director,
Statistics and Data Systems

Records are on file and available for inspection in the offices of Research Statistics in Summit, New Jersey.

REFERENCES AND NOTES:

1. Grizzle, James E. "The Two-Period Changeover Design and Its Use in Clinical Trials", Biometrics 21, (June, 1965), pp. 467-480.

2. Westlake, W.J. "Use of Confidence Intervals in Analysis of Comparative Bioavailability Trials". J. Pharm. Sci. (1972) 61, pp. 1340-1341.

3. Westlake, W.J. "The Use of Balanced Incomplete Block Designs in Comparative Bioavailability Trials". Biometrics 30, (June, 1974). pp. 319-327.

4. Because of computer memory considerations, terms in the linear model were restricted to those given in Table 2.

5. Cochran, W.G. and Cox, G.M. Experimental Designs. Wiley (1957). pp. 298-299.

6. The formulation by experiment interaction was pooled with main plot error.

Table 1

Mean Plasma Concentration by Time[1]

Hour	Acutrim	Dexatrim	Aqueous Solution
0	0.0 a	0.5 a	0.4 a
0.5	29.7 a	7.2 b	44.7 c
1	51.6 a	45.4 a,b	81.1 b
2	68.6 a	93.4 b	106.7 b
3	72.8 a	119.3 b	101.8 c
4	69.4 a	151.4 b	85.3 c
5[2]	77.4	161.7	80.2
6	76.3 a	153.9 b	63.5 a
8	75.9 a	118.2 b	46.2 c
10[2]	81.7	87.9	36.8
12	73.4 a	75.2 a	22.1 b
16	63.3 a	41.7 b	102.3 c
24	23.1 a,b	9.0 a	30.7 b
48	23.4 a	9.0 a	15.3 a
72	27.5 a	9.2 b	14.6 a,b
72.5[2]	56.6	22.6	49.2
73	71.8 a	53.2 b	65.3 a,b
74	88.3 a	109.2 b	87.4 a
75[2]	101.4	204.1	85.1
76	92.0 a	162.1 b	77.3 c
77	96.7 a	166.1 b	131.3 c
78	93.3 a	155.3 b	129.3 c
80	91.7 a	131.0 b	105.7 a
81[3]	90.9	125.7	158.0
82	96.1 a	99.1 a	148.5 b
83[2]	116.0	72.1	124.4
84	99.8 a	75.3 b	115.3 c
86[2]	92.8	49.6	81.8
88	69.1 a	41.4 b	62.9 a
96	25.0 a	9.2 b	15.1 a,b
100	12.5 a	3.8 a	7.3 a

1. Means labeled with a common letter at each time point are not significantly different ($p>.05$).

2. Data from Study 1 only. No comparisons made between means.

3. Data from Study 2 only. No comparisons made between means.

Table 2

Statistical Analysis for Plasma Concentrations

ANOVA

Source	df	SS	MS	F	p
Experiments	1	17373	17373	1.47	0.24
Subjects (Experiment)	16	188710	11794		
Periods	2	13208	6604	6.65	0.13
Period x Experiment	2	1987	993		
Formulations	2	36496	18248	21.74	0.04
Formulation x Experiment	2	1678	839		
Main plot error	28	48970	1749		
Time	23	2047525	89023	201.19	0.0001
Period x Time	46	23896	519	1.17	0.20
Formulation x Time	46	503837	10953	24.75	0.0001
Sub plot error	1102	487614	.442		

Table 3

Statistical Analysis for AUC
Day 1

ANOVA

Source	df	SS	MS	F	p
Experiments	1	238473	238473		
Subject (Experiment)	16	3892064	243254		
Periods	2	107969	53985	.63	0.61
Period x Experiment	2	170178	85089	1.35	0.28
Formulations	2	412082	206041	3.26	0.053
Formulation x Experiment	2	127953	63977	1.01	0.38
Error	27	1706313	63197		

	Mean
Acutrim	1343
Dexatrim	1598
Aqueous Solution	1364

95% Westlake Confidence Limits*

Acutrim vs. Dexatrim	±25.0%
Acutrim vs. Aqueous Solution	±13.0%
Dexatrim vs. Aqueous Solution	±27.7%

*limits based on pooled error term

Table 4

Statistical Analysis for AUC
Day 4

ANOVA

Source	df	SS	MS	F	p
Experiments	1	658321	658321		
Subject (Experiment)	16	7991833	499490		
Periods	2	756517	378259	27.67	0.03
Period x Experiment	2	27335	13668	0.13	0.88
Formulations	2	132241	66121	0.48	0.68
Formulation x Experiment	2	278046	139023	1.28	0.29
Error	28	3052864	109031		

	Mean
Acutrim	1649
Dexatrim	1732
Aqueous Solution	1831

95% Westlake Confidence Limits*

Acutrim vs. Dexatrim	±15.4%
Acutrim vs. Aqueous Solution	±19.9%
Dexatrim vs. Aqueous Solution	±15.4%

*limits based on pooled error term

FIGURE 1

MEAN PLASMA CONCENTRATION OF PHENYLPROPANOLAMINE

STUDIES ONE AND TWO COMBINED

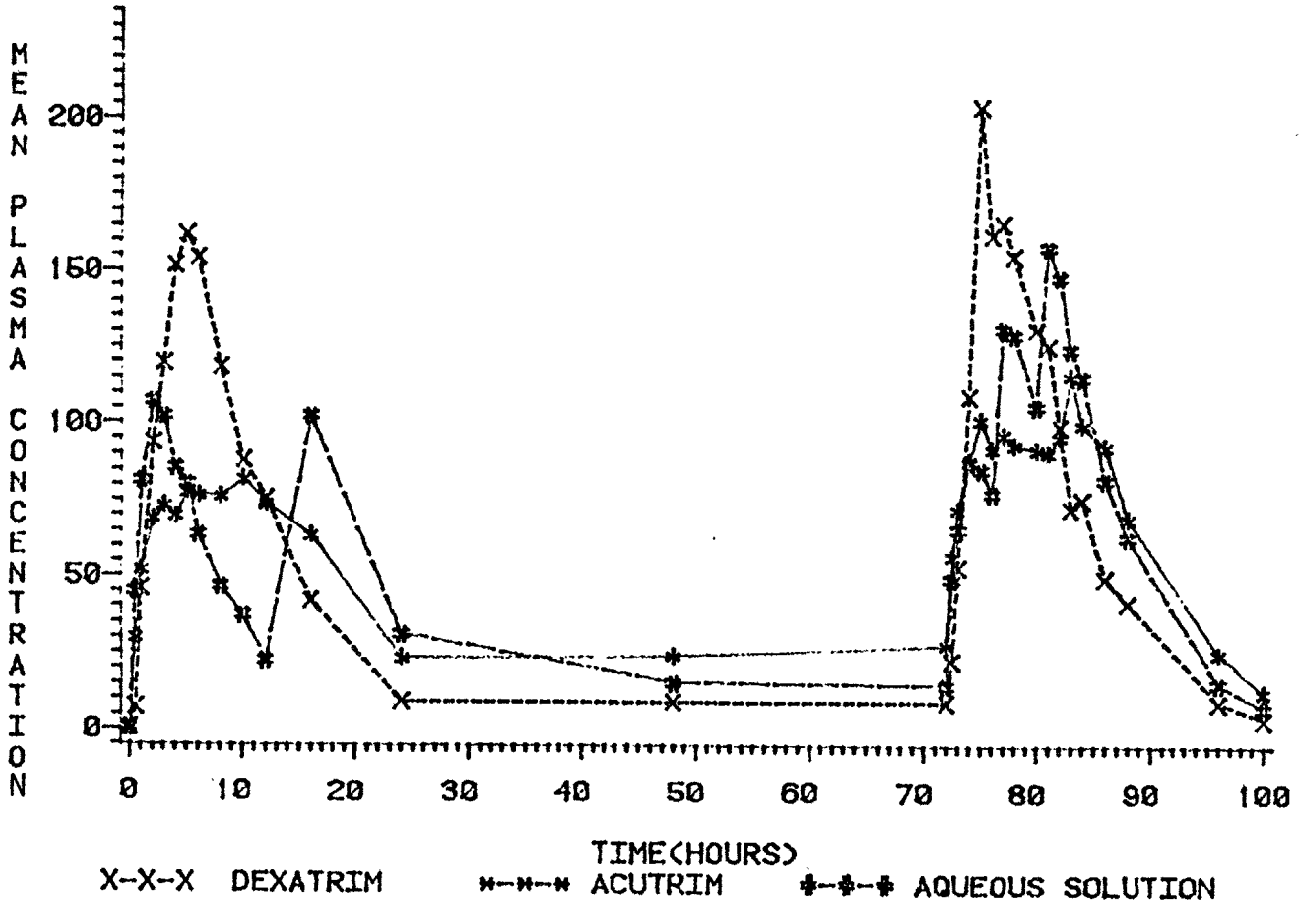


FIGURE 2

MEAN PLASMA CONCENTRATION OF PHENYLPROPANOLAMINE

DAY 1

STUDIES ONE AND TWO COMBINED

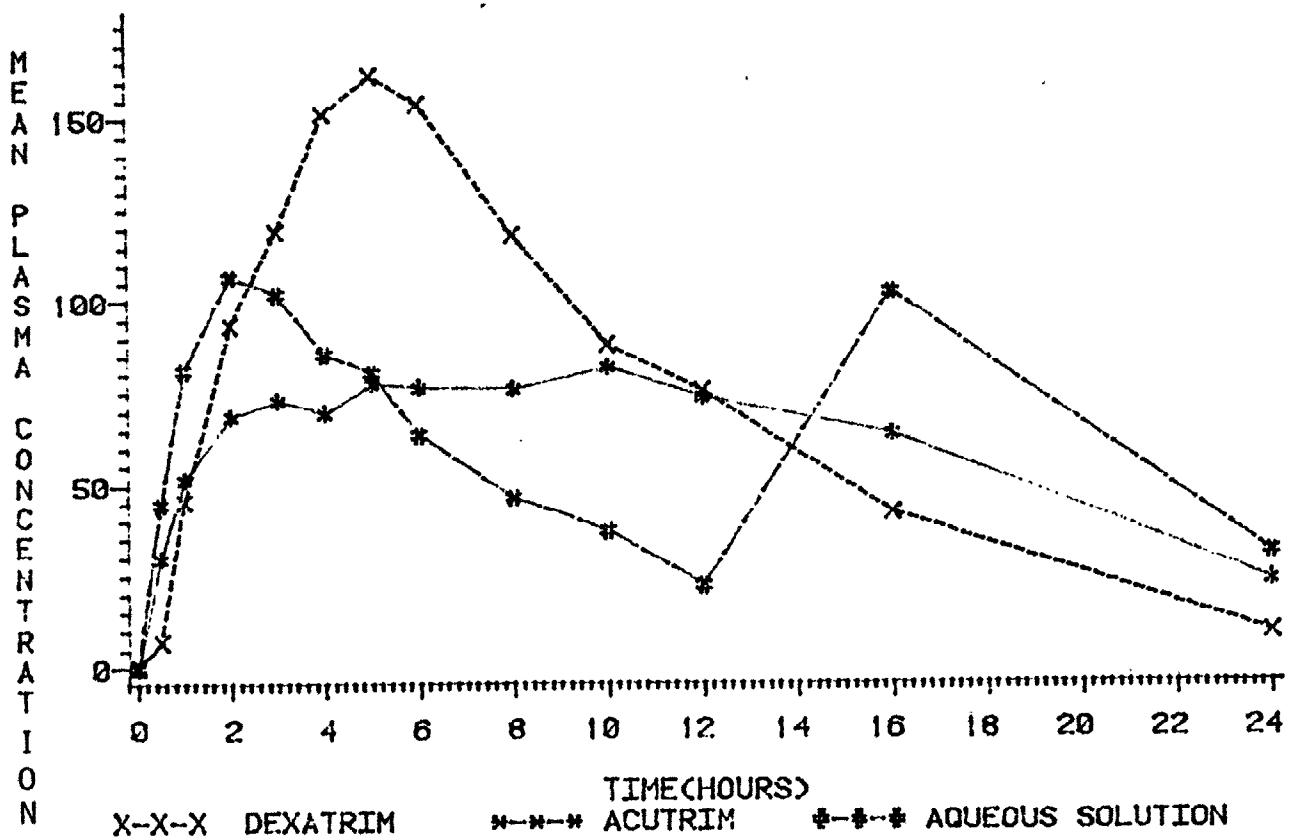
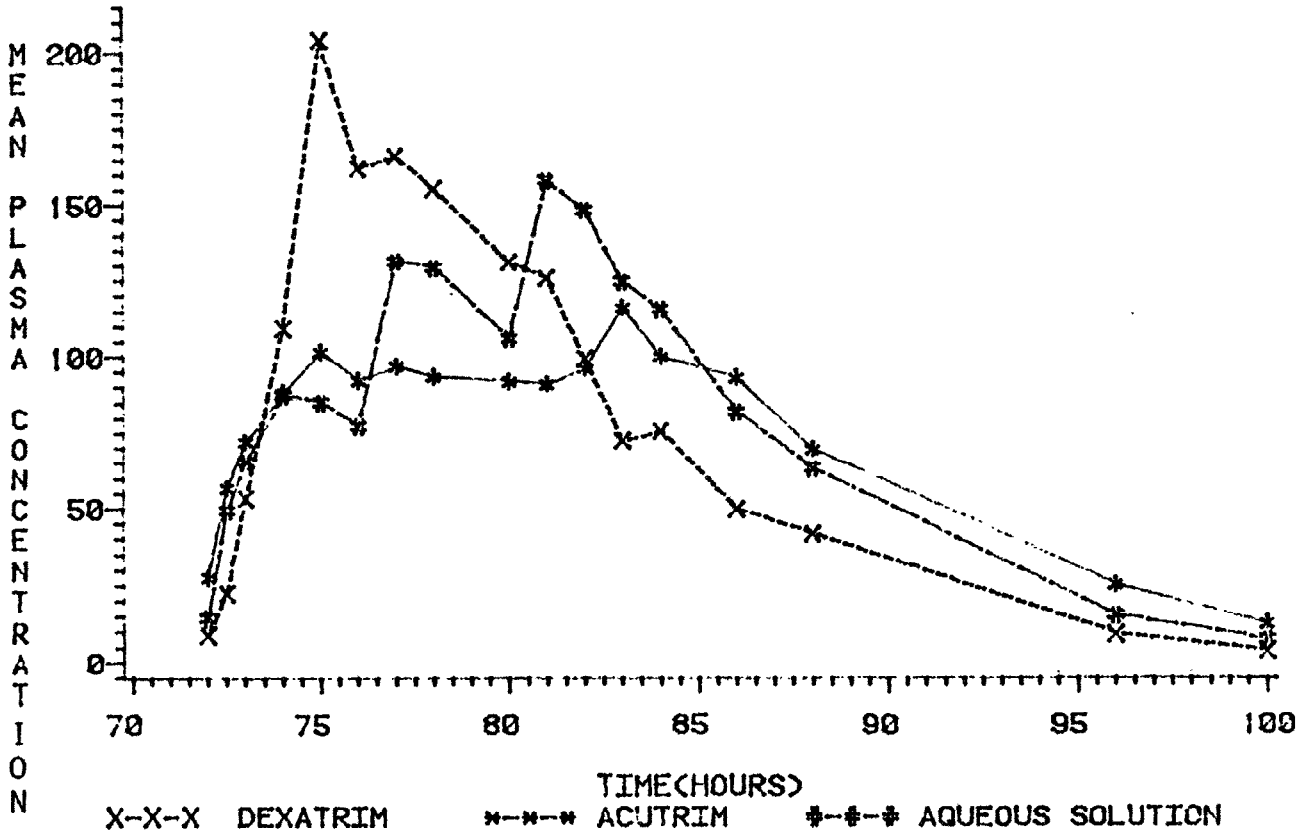


FIGURE 3

MEAN PLASMA CONCENTRATION OF PHENYLPROPANOLAMINE

DAY 4

STUDIES ONE AND TWO COMBINED



CONTENT UNIFORMITY RESULTS
(Ten Dosage Units)

Product	Lot #	Content Average (mg)	C.V.(%)	Range (mg)
Dexatrim	MDF1280A	76.4	10.2	68.7-92.0
Dexatrim	SDF282E	73.7	13.3	61.0-87.2
Dexatrim	583A	74.6	5.60	69.2-83.3
Dexatrim	583D	72.5	11.3	59.1-88.4
Acutrim	335	74.1	1.90	72.2-76.1
Acutrim	336	75.7	2.70	72.0-78.6
Acutrim	337	78.3	2.32	75.7-81.8
Acutrim	341	74.3	2.17	72.3-76.1
Acutrim	344	75.6	2.94	72.4-78.5
Acutrim	345	75.4	2.42	72.3-78.4